International Immunopharmacology 70 (2019) 378–386

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Dendritic cells treated by *Trichinella spiralis* muscle larval excretory/secretory products alleviate TNBS-induced colitis in mice

Xuemin Jin[a,1], Yong Yang[a,1], Xue Bai[a,1], Haining Shi[b], Wenbao Zhang[c], Zhuangzhi Zhang[d], Wanzhong Jia[e], Jiaojiao Lin[f], Mingyuan Liu[a,*], Xiaolei Liu[a,*]

[a] Key Laboratory of Zoonosis Research, Ministry of Education, Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun, China
[b] Mucosal Immunology Laboratory, Pediatric Gastroenterology Unit, Massachusetts General Hospital, United States of America
[c] The First Affiliated Hospital of Xinjiang Medical University, Xinjiang, China
[d] Xinjiang Veterinary Research Institute, Xinjiang Academy of Animal Sciences, Xinjiang, China
[e] Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou 730046, China
[f] Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai, China

ARTICLE INFO

Keywords:
Trichinella spiralis
Muscle larvae excretory/secretory products
Dendritic cells
TNBS
Colitis
Th2 response
Treg

ABSTRACT

Background: Therapeutic potential of helminth have been shown to have a protective effect on immune-mediated diseases such as Crohn's disease (CD), which is associated with increased production of T helper cell type 1. However, helminth therapy is unacceptable to patients due to side-effects and the fear of parasites. As helminths regulate the cellular immune responses through innate cells such as dendritic cells (DCs), cellular immunotherapy has been considered a therapeutic option to treat CD.

Methods: Bone marrow-dendritic cells were generated, enriched and treated with *Trichinella spiralis* muscle larval excretory/secretory products (*Ts*-MLES). DCs maturation was measured by flow cytometry and cytokine production of DCs were measured by ELISA. Colitis was generated by intrarectal administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) solution. For adoptive transfer, *Ts*-MLES treated-DCs injected intravenously 24 h prior to TNBS challenge. Disease activity index (DAI) including weight loss, diarrhea, and bloody stool were measured. Colon segments were stained with hematoxylin and eosin (H.E.) and periodic acid schiff (PAS) staining for histological damage scoring. The relative mRNA expression of cytokines in colon was analyzed by RT-PCR. Cytokine production in colon was measured by ELISA. Splenocytes were separated and cytokine profiles including Th1 (IFN-γ), Th2 (IL-4, IL-13), and Treg subsets (IL-10, TGF-β) were analyzed by flow cytometry.

Results: *Ts*-MLES regulated the maturation and cytokine production of DCs. *Ts*-MLES -DC ameliorated the severity of the TNBS-induced colitis. In the colon and the spleen, *Ts*-MLES-DC decreased IFN-γ (Th1) significantly and increased Th2 (IL-4, IL-13)- and Treg (IL-10, TGF-β)- related cytokines.

Conclusions: *Ts*-MLES-DC ameliorated the severity of the TNBS-induced colitis through decreasing IFN-γ. *Ts*-MLES-DC skewed the Th1-mediated response toward the Th2 type and regulatory T cell response.

1. Background

Inflammatory bowel disease (IBD) is characterized by a chronic relapsing inflammatory condition of the gastrointestinal tract. IBD primarily encompasses ulcerative colitis (UC) and Crohn's disease (CD) [1]. CD is associated with increased production of T helper cell type 1 (Th1)-like cytokines such as Interferon (IFN)-γ. Numerous epidemiological studies have shown that there is an inverse relationship between the prevalence of CD and exposure to helminthic parasites in developing countries and developed countries [2]. This relationship could be explained by the hygiene hypothesis, which suggests that the removal of the regulatory effects of parasites tends to lead to an imbalance in the immune system and an increase in immune-mediated diseases [3]. Several studies in murine models of disease and clinical trials have demonstrated the therapeutic potential of helminths and immunomodulatory helminth-derived proteins in the treatment of CD [4].

However, helminth therapy is unacceptable to patients due to side-effects and the fear of parasites. In the host, parasites regulate the cellular immune responses through innate cells, such as dendritic cells (DCs). Recently, cellular immunotherapy has been considered a

* Corresponding authors.
E-mail addresses: mingyliu666@163.com, liumy36@163.com (M. Liu), liuxlei@163.com (X. Liu).
[1] Equal contributors.

https://doi.org/10.1016/j.intimp.2019.02.028
Received 3 February 2019; Received in revised form 13 February 2019; Accepted 14 February 2019
Available online 08 March 2019
1567-5769/ © 2019 Elsevier B.V. All rights reserved.

therapeutic option to treat CD [5,6]. Immune cells treated with parasite antigens have been used to inhibit autoimmune diseases in mice [7,8]. Of those, DCs are the most potent professional antigen presenting cells (APCs) and have the ability to regulate naïve T cell responses [9]. Helminth antigens regulate the phenotypes of DCs, which are characterized by the expression of surface costimulatory molecules and the secretion of cytokines. The maturation status and cytokine production of DCs treated with helminth-derived proteins were found to contribute significantly to the modulation of the differentiation of Th1, Th2 and regulatory T cells (Tregs) [10–12]. Th1, Th2 and Tregs play important roles in controlling autoimmunity through maintaining immunologic tolerance [13].

Thus far, it has been reported that DCs treated with a crude extract of *Hymenolepis diminuta* or excretory/secretory product of *Heligmosomoides polygyrus bakeri* can suppress colitis in mice [14,15]. Crohn's disease is results from dysregulated Th1-type mucosal inflammation [16]. 2,4,6-Trinitrobenzene sulfonic acid (TNBS)-induced colitis is a well-established model of intestinal inflammation that elicits a Th1-polarized colonic immune response and expresses important histological features of human IBD [17]. Notably, infection with *Trichinella spiralis* (*T.spiralis*) alleviated experimental colitis in mice [18]. During chronic infection, *T. spiralis* releases excretory/secretory products (ESPs), which modulate the immune response for the successful survival of *T.spiralis* within the host [19]. Muscle larval ESPs of *T.spiralis* induce the immunological balance away from Th1, toward Th2 and Treg responses by modulating the DC phenotype [11]. In addition, *T.spiralis* muscle larval ESPs-(MLES) treated DCs display an increased capacity to induce regulatory T cells (Tregs). This finding indicated that DCs treated with ESPs may offer a potential therapeutic option for Th1-mediated diseases.

In this paper, phenotype of treated DC were characterized and the ability of DCs treated by *T. spiralis* muscle larval ESPs to alleviate TNBS-induced colitis in mice was investigated. We first revealed that DCs treated with muscle larval ESPs of *T. spiralis* have a protective effect on TNBS-induced colitis. The MLES -treated DCs inhibited the Th1 immune response and enhanced the Th2 and regulatory T cell immune response *in vivo*. Our results demonstrate the therapeutic potential of *T. spiralis* treated-DCs, an alternative cell-based therapy, for CD or other Th1 immune mediated diseases.

2. Materials and methods

2.1. Animals

BALB/c mice (female, 6–8 weeks old) were purchased from the Shanghai SLAC Company. Female Wistar rats were purchased from the Experimental Animal Centre of College of Basic Medical Sciences, Jilin University (Changchun, China). All animal experiments were performed according to regulations of the Administration of Affairs Concerning Experimental Animals in China. The protocol was approved by the Institutional Animal Care and Use Committee of Jilin University (20170318).

2.2. Parasites and preparation of ES

The *T. spiralis* isolate (ISS534) was obtained from a naturally infected domestic pig in Henan Province of China [20]. Briefly, Wistar rats were orally infected with 3000 infective larvae, and *T. spiralis* muscle larvae were recovered at 35 days post infection (dpi) *via* artificial digestion with pepsin-HCl (1% pepsin and 1% HCl at 37 °C for 2 h) [21]. All *T. spiralis* muscle larvae were washed three times in saline solution and were incubated separately in the pre-warmed serum-free RPMI 1640 medium containing 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C under 5% atmospheric CO_2 for 24 h. After centrifugation, the supernatant containing ES products was dialyzed and concentrated [22]. Protein concentration was determined by a Pierce bicinchoninic acid Protein Assay Kit (Thermo Scientific, Rockford, IL). We made three different preparations of ES products from muscle larvae of *T. spiralis* to perform several experiments independently. One representative experiment is shown here.

2.3. Bone marrow-derived dendritic cell (BMDC) isolation, culture, and stimulation

Bone marrow-dendritic cells (BMDCs) were generated from mouse bone marrow cells as described previously [23]. Briefly, bone marrow cells were obtained from C57BL/6 mice and cultured in RPMI 1640 medium containing growth factors of 20 ng/ml recombinant GM-CSF, 20 ng/ml IL-4 (Sigma–Aldrich) and 10% FBS at 37 °C, 5% CO_2. Immature DCs were enriched by positive selection with anti-CD11c magnetic beads (Miltenyi Biotec) according to the manufacturer's instructions. DCs were harvested on day 7 for further experiments. The enriched $CD11c^+$ DCs were typically of > 90% purity as determined by flow cytometry. To assess the effect of MLES on the phenotype of DCs, DCs were treated with MLES (50 μg/ml). To induce mature DCs, the cells were stimulated with LPS (100 ng/ml, Sigma–Aldrich) and IFN-γ (50 ng/ml, Sigma–Aldrich) *in vitro* for 48 h. The immature DCs were stimulated with sterile phosphate-buffered saline (PBS) as control. Cell culture supernatants were collected and stored at −80 °C. Cytokine levels in the supernatant were quantified by ELISA.

2.4. Induction of colitis and adoptive transfer of DCs

Colitis was generated by intrarectal administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) solution (sigma, USA). Briefly, mice were fasted for 24 h with free access to drinking water and then were pressed lightly on the abdomen to facilitate intestinal emptying and anesthetized using sodium pentobarbital (50 mg/kg, ip). Next, the mixture (5% TNBS and absolute ethyl ethanol with ratio 1:1) was administrated intrarectally *via* a flexible catheter of 3.5 cm length. The mice in control group were administrated with 50% ethyl ethanol in the same way. There are 6 mice in every group. For adoptive transfer [14], MLES treated-DCs were washed (×3) with sterile PBS, and 1×10^6 cells in 500 μl of sterile PBS were injected intravenously (i.v.) 24 h prior to TNBS challenge. Three days later, colitis was induced by TNBS in these recipient mice. Mice were humanely euthanized by CO_2, and then the colon and spleen were collected for the following experiments. The protective efficacy against TNBS challenge was determined in three independent experiments. One representative experiment is shown here.

2.5. Assessment of colitis

The mice in each group were observed daily and given a clinical disease score (disease activity index, DAI) ranging from 0 to 12 based on the clinical manifestations of weight loss, diarrhea, and bloody stool [24] (Table 1). Colonic segments ready for histopathological examination were fixed in 10% formalin, embedded in paraffin, carefully sectioned at 5 μm thickness and stained with hematoxylin and eosin (H.E.), and were stained with periodic acid Schiff (PAS) staining according to standard protocols. The histological damage scoring was applied to grade the severity of inflammation based on the following 2

Table 1
Disease activity index score parameters (DAI).

Weight loss (%)	Stool	Bloody stool	Index
0–1%	Normal	None	0
1–4%	Soft and shaped	Between	1
4–8%	Loose	Slight	2
8–12%	Between	Between	3
> 12%	Diarrhea	Gross bleeding	4

parameters: epithelial lesion (0, none damage; 1, some loss of goblet cells; 2, extensive loss of goblet cells; 3, some loss of crypts; 4, extensive loss of crypts); infiltration (0, none infiltration; 1, infiltration around crypt bases; 2, infiltration spreading to muscularis mucosa; 3, extensive infiltration in the muscularis mucosa with abundant oedema; 4, infiltration spreading to submucosa). The total histological grade ranged from a minimum of 0 to a maximum of 8 as stated above [24].

2.6. MPO activity assay

Myeloperoxidase (MPO) activity was determined by using a MPO assay kit (Nanjing Jiancheng Bio-engineering Institute, China) to detect the degree of myeloid cell infiltration in the colon. Briefly, 100 mg colon tissues were cut, followed by homogenizing in solution containing 0.9 ml of saline, pH 6.0, 0.5% hexadecyl trimethyl ammonium hydroxide, and then centrifuged at 12,000 g (4 °C) for 15 min. The protein concentration of the colon homogenate supernatants was determined using a Bicinchoninic Acid Protein Assay Kit (Beyotime Biotechnology, China). One hundred microliters of the supernatants were collected and went on following manufacturer's instructions. The MPO activity of the supernatants was determined and expressed as units per gram of total protein (U/g).

2.7. Flow cytometry staining

The stimulated DCs were stained with a FITC-conjugated monoclonal antibody (mAb) to CD11c, and PE-conjugated mAbs to CD40, CD80 or CD86 (Biolegend, USA). Splenocytes were separated from spleens of mice and suspended in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin. To determine Treg subset levels, cells were first preincubated with Fc Block (anti-Mouse CD16/CD32, BD Biosciences) for 15 min to reduce the nonspecific binding of the labeled antibodies and then, some of the cells were stained with FITC-anti-CD4 and APC-anti-CD25 antibodies, fixed and permeabilized using a FIX/PERM set (BD Biosciences). Cells were then blocked in 5% rat serum prior to intracellular staining with a PerCP-Cy5.5-labeled anti-Foxp3 antibody.

For T helper-related cytokines expression, cells were stained for surface markers (PerCP-Cy5.5-anti-CD3 and FITC-anti-CD4 antibodies, BD Biosciences) for 35 min at 4 °C in the dark. These cells were fixed, permeabilized using a FIX/PERM set (Biolegend) and blocked in 5% rat serum for 10 min at room temperature prior to intracellular staining with APC-conjugated mAbs to IFN-γ, IL-4 or IL-13.

2.8. RNA extraction and real time (RT)-PCR

mRNA expression levels were quantified using RT-PCR. Briefly, RNA extraction was performed by lysing 100 mg of mouse colon tissue samples with Trizol reagent (Invitrogen), according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from 1.0 μg of total RNA with oligo (dT) primers using a Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA), according to the manufacturer's protocol, and RT-PCR was performed using SYBR Green QPCR Master Mix (TaKaRa, Japan), according to manufacturer's instructions. The primers used for RT-PCR are listed in Table 2. PCR was performed with a reaction mixture with a total volume of 25 μl comprising the following: 12.5 μl SYBR® Premix Ex TaqTM (2×), 1 μl forward primer (10 μmol/l), 1 μl reverse primer (10 μmol/l), 2 μl template, and 8.5 μl ddH_2O. The reaction comprised the following steps: an initial denaturation at 95 °C for 5 min, followed by amplification for 45 cycles at 95 °C for 15 s and 60 °C for 60 s. These steps were performed with an Applied Bioscience 7500 thermocycler and FastStart Universal SYBR Green Master (Roche Applied Science, Germany). The relative mRNA expression levels of the target genes were normalized to those of the indicated housekeeping gene (GAPDH) and were quantified using the comparative Ct method and the formula $2^{-\Delta\Delta CT}$ [25].

Table 2
The primers of quantitative RT-PCR.

Genes	Primer	Sequence (5′ → 3′)
IFN-γ	Forward primer	GGAACTGGCAAAAGGATGGTGAC
	Reverse primer	GCTGGACCTGTGGGTTGTTGAC
IL-4	Forward primer	CTGTAGGGCTTCCAAGGTGCTTCG
	Reverse primer	CCATTTGCATGATGATGCTCTTTAGGC
IL-13	Forward primer	GCTTGCCTTGGTGGTCTCGCC
	Reverse primer	GGGCTACACAGAACCCGCCA
IL-10	Forward primer	AGCCGGGAAGACAATAACTG
	Reverse primer	CATTTCCGATAAGGCTTGG
TGF-β	Forward primer	AACTATTGCTTCAGCTCCACAG
	Reverse primer	AGTTGGCATGGTAGCCCTTG
GAPDH	Forward primer	ACTCCACTCACGGCAAATTC
	Reverse primer	TCTCCATGGTGGTGAAGACA

2.9. Determination of colon cytokines

A segment of the colon was excised and washed twice in clean phosphate-buffered saline containing penicillin and streptomycin. Then, the colon was further cut into 1 cm^2 pieces and placed in 24-well flat bottom-well culture plates with 1 ml RPMI 1640 supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C, 5% CO_2 for 24 h. The supernatant was collected, and cellular debris was removed by centrifugation. Cytokine levels in the supernatant were quantified by ELISA [26,27].

2.10. Statistical analysis

All results are expressed as the mean ± SD. Statistical analysis was performed using the GraphPad Prism 5 software for Windows. One-way and two-way analysis of variance (ANOVA) were used to compare significant differences between different conditions. p values are expressed as $^{*}p < .05$, $^{**}p < .01$ and $^{***}p < .001$.

3. Results

3.1. T. spiralis MLES (Ts-MLES) regulated DCs phenotype

To investigate the ability of the *Ts*-MLES to regulate the maturation of DCs, surface costimulatory molecules on $CD11c^+$ DCs (> 90% $CD11c^+$, Fig. 1A) were measured. As shown in Fig. 1B and C, the *Ts*-MLES-treated group showed small increases in CD40, CD80 and CD86 expression compared to the PBS control group. In the absence of *Ts*-MLES, surface markers (CD40, CD80 and CD86) on $CD11c^+$ DCs stimulated by LPS\IFN-γ were upregulated. However, the *Ts*-MLES-treated group had significantly inhibited upregulation of CD40, CD80 and CD86 expression on DCs compared to the LPS\IFN-γ group, indicating that *Ts*-MLES inhibits LPS\IFN-γ-induced maturation (Fig. 1B). These results suggested that *Ts*-MLES-treated DCs (*Ts*-MLES-DC) were regulated to semimaturation. Cytokine production secreted by DCs was examined. LPS\IFN-γ significantly elevated the IL-12 production by DCs, but this production was inhibited by *Ts*-MLES. In addition, IL-10 and TGF-β levels were significantly enhanced by *Ts*-MLES compared to LPS\IFN-γ or PBS (Fig. 2).

3.2. Ts-MLES-DC alleviated TNBS-induced colitis in mice

To investigate the protective effect of *Ts*-MLES-DC on colitis, we established a model of TNBS-induced mouse colitis. The DAI scores observed in the TNBS group were significantly elevated 3 days after induction (Fig. 3A). The rate of body weight loss of the TNBS-induced mice was > 12% after 3 days. The mice treated with *Ts*-MLES-DC prior

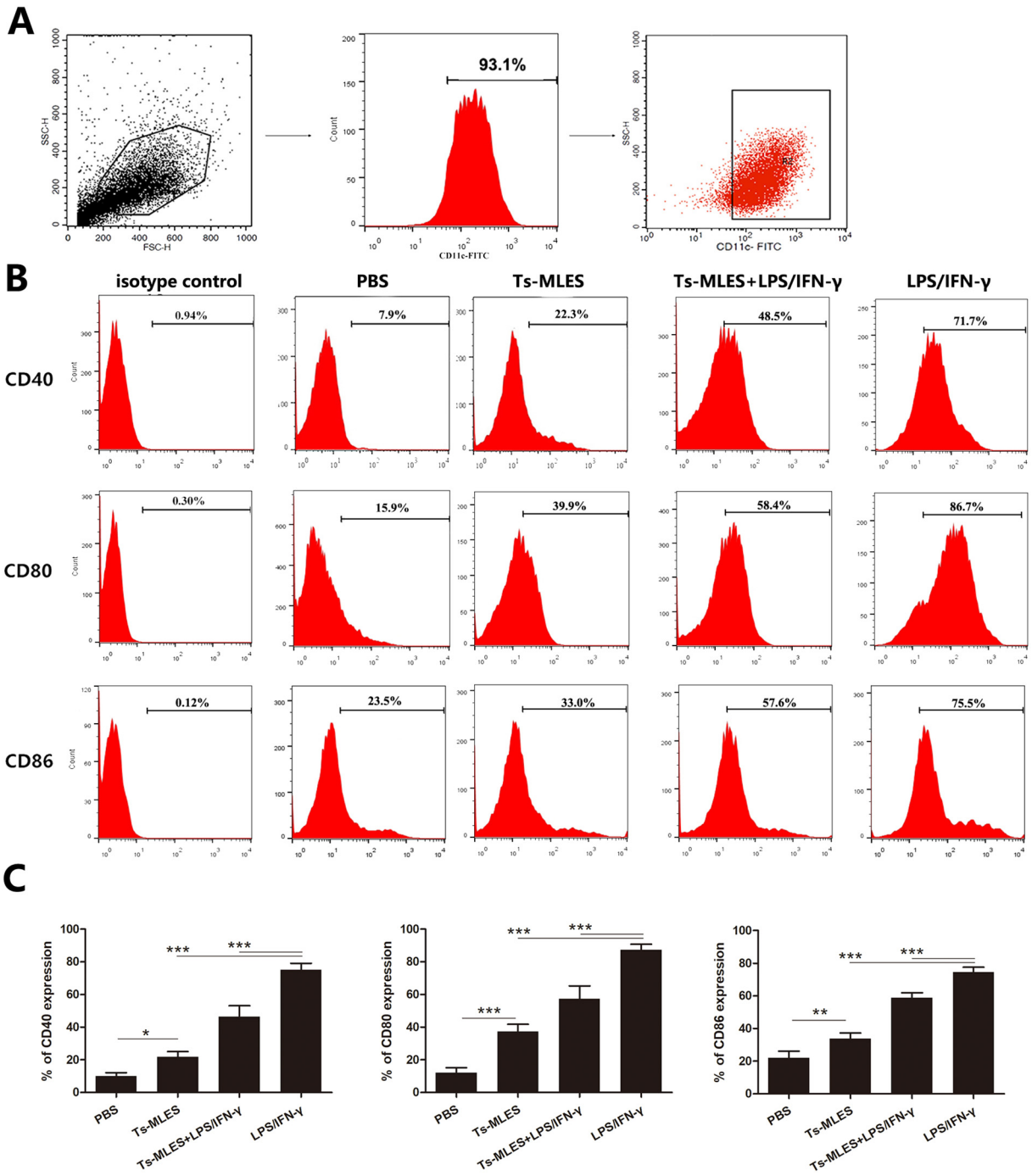

Fig. 1. Expression of surface costimulatory molecules on DCs induced by *Ts*-MLES. DCs were treated with *Ts*-MLES (50 μg/ml). To induce mature DCs, the cells were stimulated with LPS (100 ng/ml, Sigma–Aldrich) and IFN-γ (50 ng/ml, Sigma–Aldrich) *in vitro* for 48 h. The stimulated DCs were stained with a FITC-conjugated monoclonal antibody (mAb) to CD11c, and PE-conjugated mAbs to CD40, CD80 or CD86 respectively. (A) The percentage of $CD11c^+$ DCs were measured by FACS. (B) The expression levels of CD40, CD80 and CD86 were measured by FACS. Data represent means ± standard deviations (SD) of each group ($n = 3$) of the results from three individual experiments * $p < .05$, ** $p < .01$, *** $p < .001$ as indicated by line (one-way ANOVA with Tukey's posttest). These figures are representative of three independent experiments.

to TNBS challenge displayed a significantly decreased body weight loss on days 2 and 3 after TNBS challenge (Fig. 3B). The TNBS group typically showed colonic shortening, whereas colon length was significantly increased in the *Ts*-MLES-DC group (Fig. 3C and D). In addition, *Ts*-MLES-DC treatment significantly reduced the level of TNBS-induced hyperactivated MPO (TNBS: 1.467 ± 0.145; *Ts*-MLES-DC + TNBS: 0.900 ± 0.185) (Fig. 3E). Moreover, histopathological changes in the colon were further analyzed. The TNBS group exhibited a loss of goblet cells, distortion of the crypts and extensive infiltration. However, administration of *Ts*-MLES-DC obviously improved the pathological changes observed by microscopic analysis (Fig. 4). These results suggested that the adoptive transfer of *Ts*-MLES-DC attenuated the severity of TNBS-induced colitis.

3.3. *Ts-MLES-DC regulated T cell differentiation in vivo*

The splenocytes were isolated and assessed by flow cytometry. The population of $CD3^+$ $CD4^+$ IFN-γ^+ T cells associated with the Th1

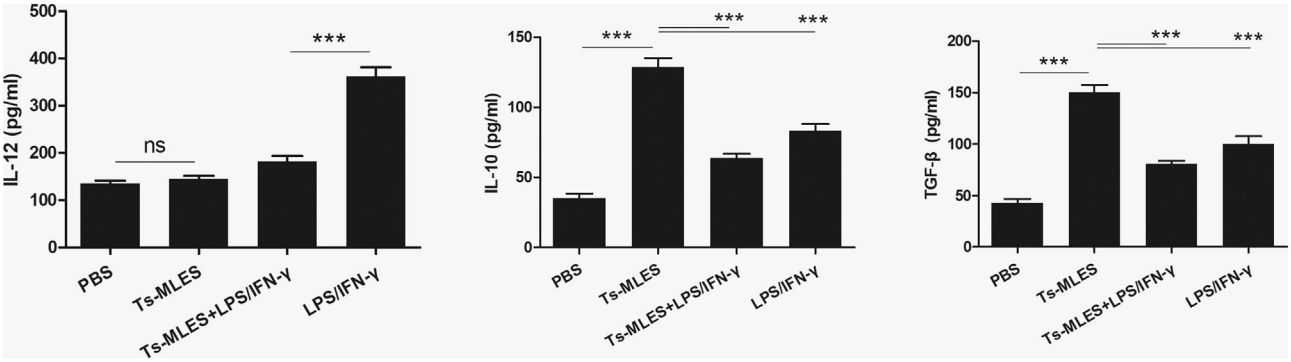

Fig. 2. Cytokine production by DCs treated with *Ts*-MLES. DCs were cultured for 48 h and the supernatants were measured by ELISA. The results are presented as the mean ± SD of each group ($n = 3$) from three individual experiments. * $p < .05$, ** $p < .01$, *** $p < .001$ as indicated by line (one-way ANOVA with Tukey's posttest).

immune response was significantly enhanced in the TNBS-induced mice compared to the control group (Fig. 5A). Compared to the TNBS group, the group treated with *Ts*-MLES-DC before colitis induction had significantly decreased populations of Th1 cells (Fig. 5A) and increased populations of $CD3^+$ $CD4^+$ $IL\text{-}4^+$ cells and $CD3^+$ $CD4^+$ $IL\text{-}13^+$ cells, which were defined as Th2 cells (Fig. 5B, C). The percentage of Tregs was also measured. The results showed a significant decrease in $CD4^+$ $CD25^+$ $Foxp3^+$ T cells in TNBS-induced mice, but an increase in the Treg population was observed in the mice who were administered *Ts*-MLES-DC (Fig. 5D).

Moreover, the colon tisuee and culture supernatant was used to determine cytokine production by RT-PCR and ELISA. RT-PCR results showed that IL-4, IL-13, IL10 and TGF-β expression levels were significantly up-regulated by treatment with *Ts*-MLES-DC prior to TNBS-induced colitis (Fig. 6A). The relative mRNA expression and the level of IFN-γ in the colon was significantly increased in the TNBS-induced mice (1118.0 ± 90.05 pg/ml) but was significantly decreased in the *Ts*-MLES-DC-treated mice (662.5 ± 42.50 pg/ml). IL-4 (128.1 ± 11.54 pg/ml) and IL-13 (1210.2 ± 137.6 pg/ml) levels were enhanced when mice were administered TNBS alone, and *Ts*-MLES-DC-treated group also had significantly higher levels of these two cytokines (IL-4: 213.5 ± 14.33 pg/ml; IL-13: 4348.1 ± 444.20 pg/ml) associated with the Th2 immune response compared to the TNBS group. Furthermore, compared to the control group and TNBS group, the *Ts*-MLES-DC-treated group had higher levels of IL-10 (1019.1 ± 40.67 pg/ml) and TGF-β (502.5 ± 71.22 pg/ml) (Fig. 6B).

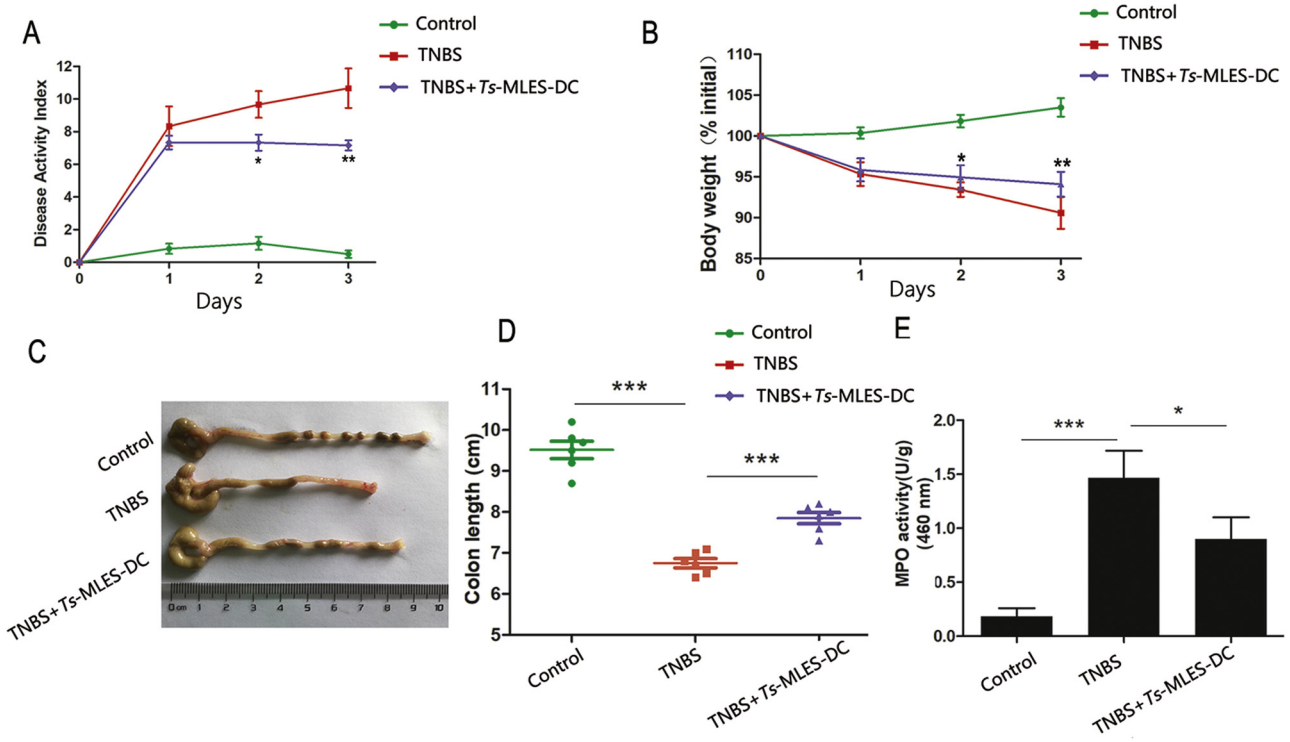

Fig. 3. Effect of *Ts*-MLES-DC on TNBS-induced colitis. For adoptive transfer, *Ts*-MLES-treated DCs a concentration of 1×10^6 cells in 500 μl sterile PBS were injected intravenously (i.v.) 24 h prior to TNBS challenge. The protective efficacy against TNBS challenge was determined in three independent experiments. One representative experiment is shown here. (A) Disease activity index (DAI) was measured during the disease process. (B) The daily mean weight change in each group was calculated (Day 2, $p < .05$, Day 3, $p < .01$, TNBS *vs* TNBS + *Ts*-MLES-DC). (C) (D) After 3 days, colons were removed, and the lengths of their colons were measured and recorded. (E) Myeloperoxidase (MPO) activity in the colonic tissues was detected. The results are representative of at least three independent experiments and expressed as the mean ± SD of each group ($n = 6$). * $p < .05$, ** $p < .01$, *** $p < .001$ as indicated by line (one-way ANOVA with Tukey's posttest) on the same day.

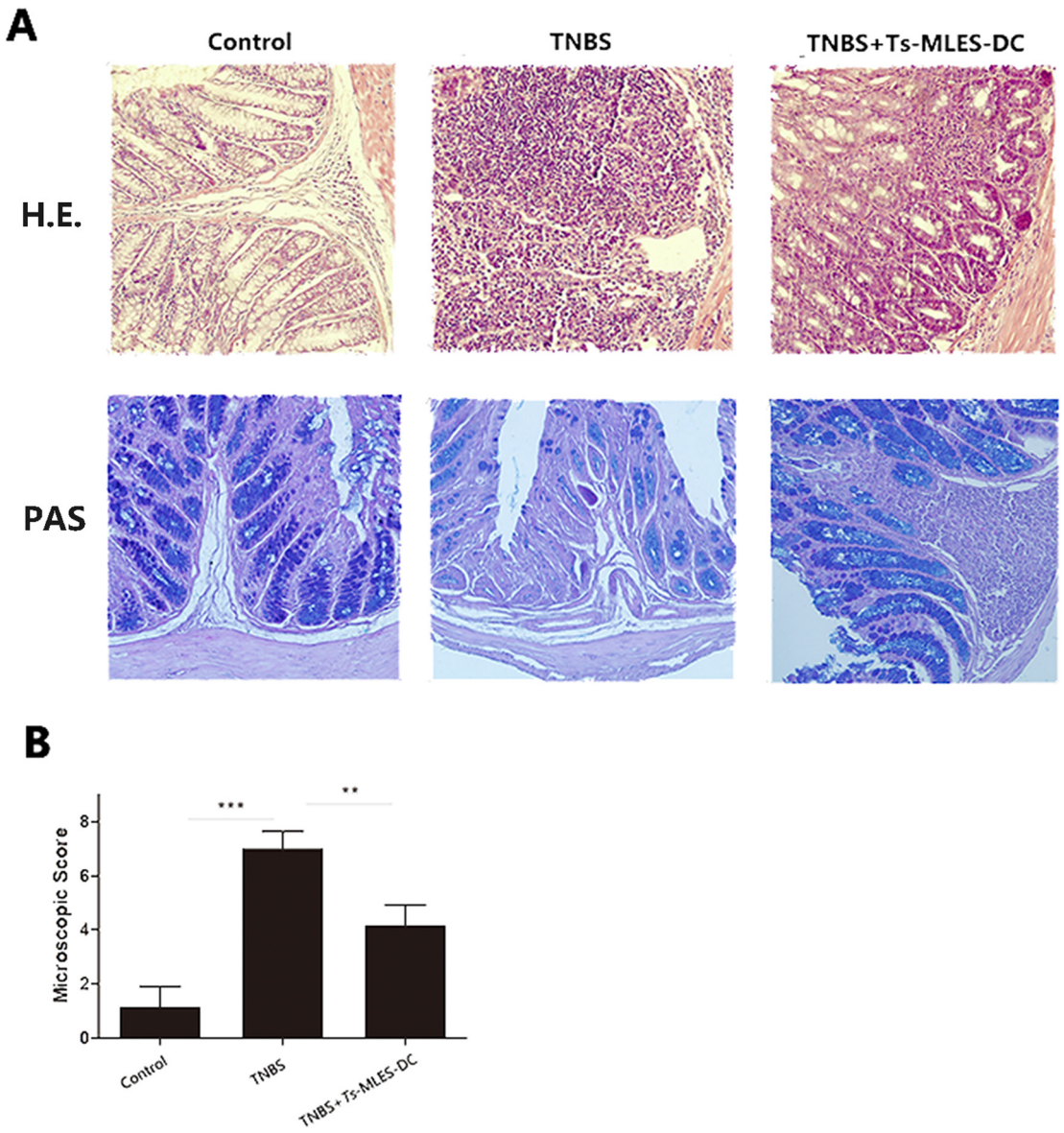

Fig. 4. Histopathological changes in colon. The colonic segments were stained with hematoxylin and eosin (H.E.), and were stained with periodic acid Schiff (PAS) staining according to standard protocols. (A) The colons from each experimental group ($n = 6$) were processed for histological evaluation (200×). (B) Histopathological damage scores were determined for the colon tissue samples. * $p < .05$, ** $p < .01$, *** $p < .001$ as indicated by line (one-way ANOVA with Tukey's posttest). These figures are representative of three independent experiments.

4. Discussion

Inflammatory bowel disease (IBD) is a chronic dysregulated inflammatory disease of intestinal tract. The patients frequently experience continuous or intermittent diarrhea, abdominal pain, rectal bleeding and fatigue due to aberrant intestinal inflammation, probably resulting from inappropriately vigorous immune responses to components of the natural intestinal fecal stream [28]. The available treatments for the disease are far from optimal. Several studies have demonstrated the therapeutic potential of helminths and immunomodulatory helminth-derived proteins in the treatment of colitis [29–31].

However, helminth therapy is unacceptable to patients due to side-effects and the fear of parasites. Recently, cellular immunotherapy has been considered a therapeutic option to treat colitis [5,6]. DCs are the major antigen presenting cells (APC) that play a relevant role in the activation of naive T lymphocytes [9]. Helminth antigens regulate the phenotypes of DCs, which are characterized by the expression of surface costimulatory molecules and the secretion of cytokines. Our results showed that semi-mature DCs were induced by *Ts*-MLES and that these DCs released cytokines that may influence the T cell response. IL-10 and TGF-β, anti-inflammatory cytokines, levels were increased in DCs treated with *Ts*-MLES. IL-10 cytokine production in DCs has been previously related to Th2 differentiation [32], and IL-10-producing DCs are critical for the polarization of naïve T cells into Tregs [33]. IL-10 and TGF-β have been shown to maintain the tolerogenic function of DCs and the suppressive abilities of IL-10-producing Tregs [34]. In addition, we observed that DCs treated with *Ts*-MLES were able to inhibit the increase in IL-12 expression induced by LPS/IFN-γ. The observed low production of IL-12 and upregulation of IL-10 by *Ts*-MLES-treated-DCs might be related to the expansion of Tregs, as shown previously [35]. It has been reported that *Ts*-MLES induce the immunological balance to shift away from the Th1 response, toward Th2 and regulatory responses by modulating DC phenotypes [11]. The Th2-type response and regulatory T cell response play roles in controlling autoimmunity. Our findings indicated that *Ts*-MLES-DC may have therapeutic potential, as

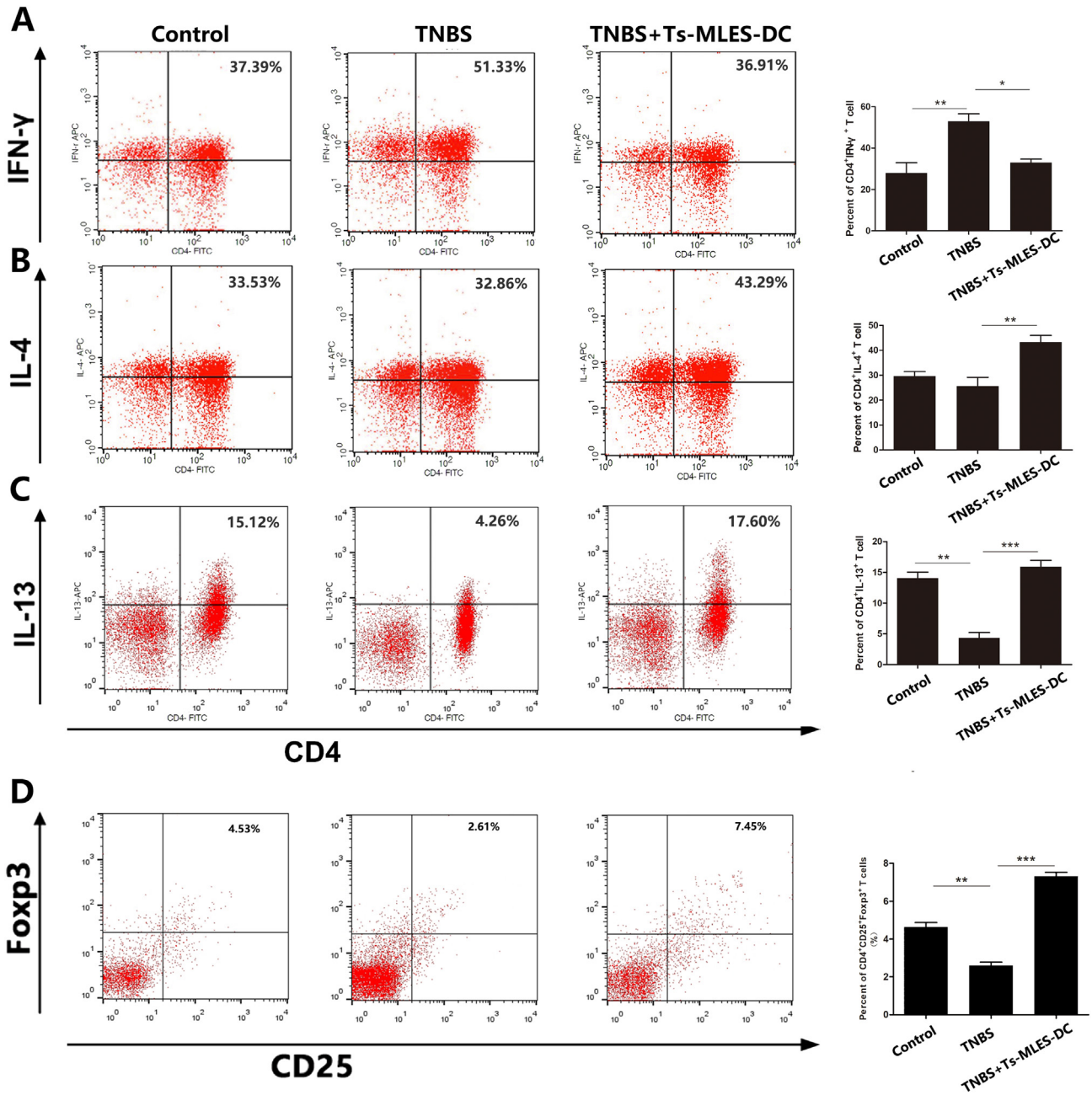

Fig. 5. Populations of $CD3^+$ $CD4^+$ T cells in the total splenocyte population. Splenocytes were separated from the spleen. (A) Populations of Th1 cells ($CD3^+$ $CD4^+$ IFN-γ^+ T cells) are shown. (B) (C) Populations of Th2 cells ($CD3^+$ $CD4^+$ IL-4^+ and IL-13^+ T cells) are shown. (D) Populations of Treg cells ($CD4^+CD25^+$ $Foxp3^+$ T cells) are shown. Data are shown as the means ± SD (three independent experiments) of each group ($n = 3$). * $p < .05$, ** $p < .01$, *** $p < .001$ as indicated by line (one-way ANOVA with Tukey's posttest). These figures are representative of three independent experiments.

an alternative cell-based therapy, for IBD or other Th1 immune mediated diseases. Furthermore, we assessed whether DC treated with ES products of *T. spiralis* protects against the development of TNBS-induced colitis. The results revealed that the DAI, body weight loss, splenomegaly and MPO activity levels were significantly diminished in TNBS-induced mice colitis administrated with *Ts*-MLES-DC. And the *Ts*-MLES-DC improved the colon lengths compared to the TNBS group. In addition, microscopic analysis demonstrated that the loss of goblet cells, distortion of crypts and extensive infiltration in TNBS-induced colitis were ameliorated through the administration of *Ts*-MLES-DC before TNBS challenge. Together, these data suggested that the adoptive transfer of *Ts*-MLES-DC alleviated the clinical disease activity in TNBS-induced colitis mice.

TNBS-induced colitis is mainly mediated by an imbalance in the T cell compartment and $CD4^+$T cells are predominantly involved in the pathogenic process [36]. While the increase in $CD3^+$ $CD4^+$ IFN-γ^+ T cells in TNBS-induced mice was inhibited by MLES-DC, the $CD3^+$ $CD4^+$ IL-4^+ T cells and $CD3^+$ $CD4^+$ IL-13^+ T cells associated with Th2-type response were enhanced by *Ts*-MLES-DC in the spleen. In the colon, by RT-PCR and ELISA, we observed similar results of cytokine production, which indicated that *Ts*-MLES-DC skewed the Th1-mediated response toward the Th2 type response *in vivo*. These results were consistent with the finding that *Ts*-MLES induced the immunological balance away from the Th1 response, toward the Th2 response by modulating DC phenotypes *in vitro* [11]. IL-4 and IL-13, which signal through IL-4 receptor alpha (IL-4Rα), play a role in maintaining Th2 immune response [37]. It has been reported that the suppression of colitis by adoptive transfer of helminth antigen-treated dendritic cells requires IL-4Rα

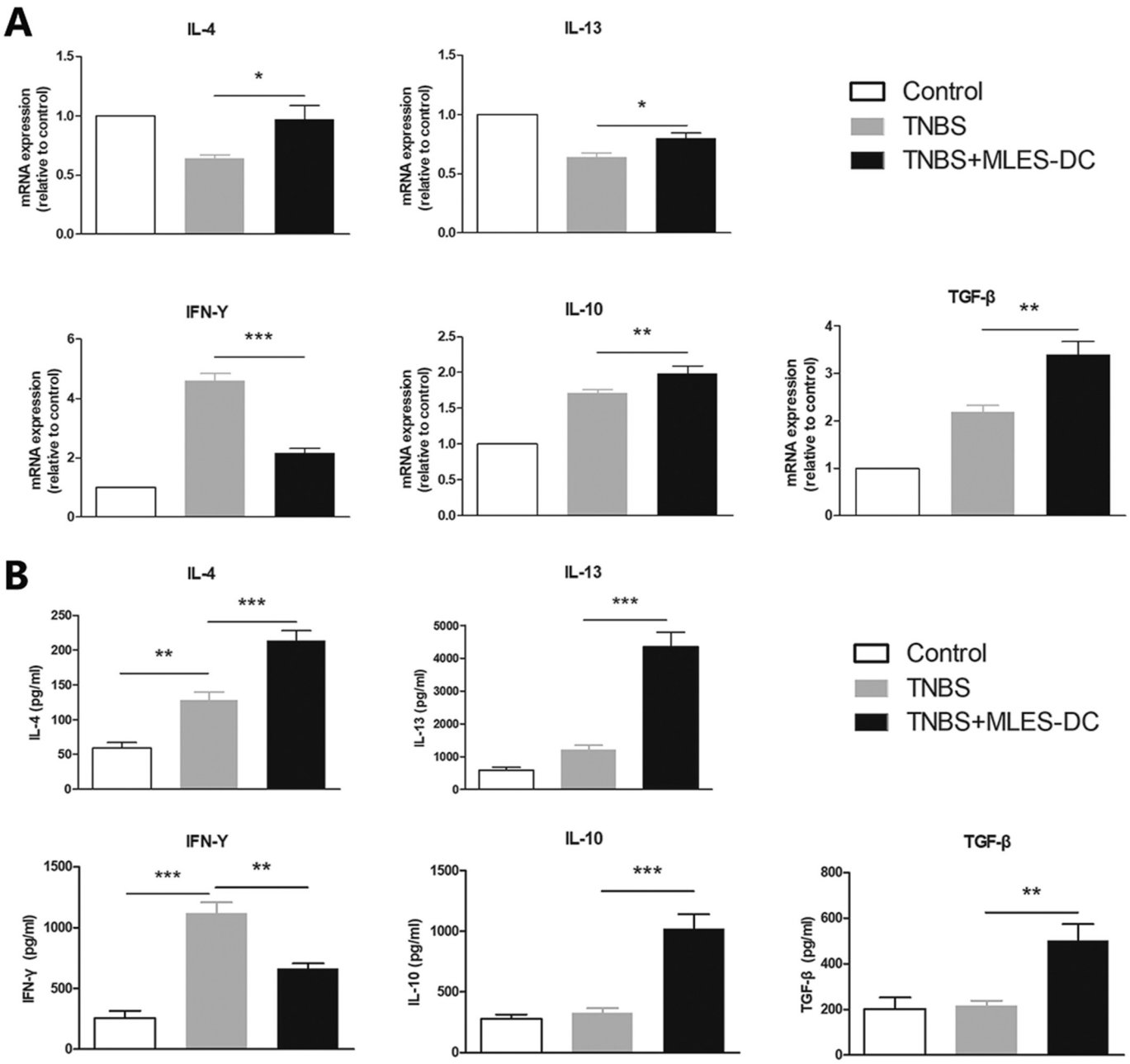

Fig. 6. Cytokine production in the colon. (A)The relative mRNA expression levels of the inflammatory cytokines IFN-γ, IL-4, IL-13, IL-10 and TGF-β in colon tissue were determined by RT-PCR, and the housekeeping gene GAPDH was used as an internal reference. (B) The colon culture supernatant was used to determine the cytokine production. IFN-γ, IL-4, IL-13, IL-10 and TGF-β levels were measured by ELISA. Data are shown as the means ± SD (three independent experiments) of each group ($n = 3$). * $p < .05$, ** $p < .01$, *** $p < .001$ as indicated by line (one-way ANOVA with Tukey's posttest).

signaling [14]. It will also be interesting to assess whether the effect of *Ts*-MLES-DC on colitis may require IL-4Rα. Moreover, several studies, showed that ES of *T. spiralis* muscle larvae, or its components possess the ability to induce the semi-mature DCs, which can induce the expansion of Tregs *in vitro* [11,38]. We demonstrated that not only the percent of Tregs in total splenocyte population but also the levels of IL-10 and TGF-β in the colon supernatant were significantly increased when an adoptive transfer of *Ts*-MLES-DC was performed prior to TNBS-induced colitis. Tregs are a fundamental mechanism of immune regulation during helminth infections, and the induction of $Foxp3^+$ Treg responses is of principal importance for the design of both prophylactic helminth treatments and therapies for autoimmunity [39]. In host, *Heligmosomoides polygyrus* secreting products can expand Tregs population by activating TGF-β signaling *via* binding to TGF-β receptors and inducing mouse and human Foxp3+ Tregs [40]. The pathways involved in the induction of Tregs by *Ts*-MLES-DC require further studies.

5. Conclusion

We found that the administration of *Ts*-MLES-DC alleviated the severity of TNBS-induced colitis in mice. Moreover, our data demonstrated that *Ts*-MLES-DC modulated inflammatory cytokine production of colon, which resulted in ameliorating intestinal inflammation. The results suggested that *Ts*-MLES-DC inhibited Th1 immune response while enhancing the Th2 and Treg immune response in TNBS-induced colitis *in vivo*. These results provide evidence that *Ts*-MLES-DCs have the potential to mitigate CD *via* regulation of T cell responses in the

host. Thus, *Ts*-MLES-treated DCs have the potential in cell-based therapeutic option for treating IBD and other autoimmune disorders.

Conflicts of interest

Authors declare no conflict of interest.

Acknowledgements

We thank Xinrui Wang for the technical assistance. Our thanks are also extended to express our gratitude to all the people who made this work.

Funding

This study was supported by The National Key Research and Development Program of China (2017YFC1601206, 2017YFD0501302, 2017YFD0501300); Jilin Provincial Science and Technology Development Project (20180520042JH); National Natural Science Foundation of China (NSFC 31520103916).

References

[1] R.S. Blumberg, W. Strober, Prospects for research in inflammatory bowel disease, Jama 285 (2001) 643–647.
[2] J.V. Weinstock, D.E. Elliott, Helminths and the IBD hygiene hypothesis, Inflamm. Bowel Dis. 15 (2009) 128–133.
[3] L.J. Wammes, H. Mpairwe, A.M. Elliott, M. Yazdanbakhsh, Helminth therapy or elimination: epidemiological, immunological, and clinical considerations, Lancet Infect. Dis. 14 (2014) 1150–1162.
[4] J.V. Weinstock, D.E. Elliott, Translatability of helminth therapy in inflammatory bowel diseases, Int. J. Parasitol. 43 (2013) 245–251.
[5] A.E. Pedersen, E.G.W. Schmidt, M. Gad, S.S. Poulsen, M.H. Claesson, Dexamethasone/1α-25-dihydroxyvitamin D3-treated dendritic cells suppress colitis in the SCID T-cell transfer model, Immunology 127 (2009) 354–364.
[6] M.M. Hunter, A. Wang, K.S. Parhar, M.J. Johnston, N. Van Rooijen, P.L. Beck, et al., In vitro-derived alternatively activated macrophages reduce colonic inflammation in mice, Gastroenterology 138 (2010) 1395–1405.
[7] A.M. Blum, L. Hang, T. Setiawan, J.P. Urban Jr., K.M. Stoyanoff, J. Leung, et al., Heligmosomoides polygyrus bakeri induces tolerogenic dendritic cells that block colitis and prevent antigen-specific gut T cell responses, Journal of immunology (Baltimore, Md: 1950) 189 (2012) 2512–2520.
[8] C.E. Matisz, G. Leung, J.L. Reyes, A. Wang, K.A. Sharkey, D.M. McKay, Adoptive transfer of helminth antigen-pulsed dendritic cells protects against the development of experimental colitis in mice, Eur. J. Immunol. 45 (2015) 3126–3139.
[9] J. Zhu, H. Yamane, W.E. Paul, Differentiation of effector CD4 T cell populations, Annu. Rev. Immunol. 28 (2010) 445–489.
[10] L. Ma, D. Li, C. Yuan, X. Zhang, N. Ta, X. Zhao, et al., SjCRT, a recombinant Schistosoma japonicum calreticulin, induces maturation of dendritic cells and a Th1-polarized immune response in mice, Parasit. Vectors 10 (2017) 570.
[11] N. Ilic, A. Gruden-Movsesijan, J. Cvetkovic, S. Tomic, D.B. Vucevic, C. Aranzamendi, et al., Trichinella spiralis excretory-secretory products induce tolerogenic properties in human dendritic cells via toll-like receptors 2 and 4, Front. Immunol. 9 (2018) 11.
[12] Y. Xiao, M. Shi, Q. Qiu, M. Huang, S. Zeng, Y. Zou, et al., Piperlongumine suppresses dendritic cell maturation by reducing production of reactive oxygen species and has therapeutic potential for rheumatoid arthritis, J. Immunol. 196 (2016) 4925–4934.
[13] D.A.A. Vignali, L.W. Collison, C.J. Workman, How regulatory T cells work, Nat. Rev. Immunol. 8 (2008) 523.
[14] C.E. Matisz, B. Faz-Lopez, E. Thomson, A. Al Rajabi, F. Lopes, L.I. Terrazas, et al., Suppression of colitis by adoptive transfer of helminth antigen-treated dendritic cells requires interleukin-4 receptor-alpha signaling, Sci. Rep. 7 (2017) 40631.
[15] L. Hang, A.M. Blum, S. Kumar, J.F. Urban Jr., M. Mitreva, T.G. Geary, et al., Downregulation of the Syk signaling pathway in intestinal dendritic cells is sufficient to induce dendritic cells that inhibit colitis, J. Immunol. 197 (2016) 2948–2957.
[16] F. Shanahan, Crohn's disease, Lancet 359 (2002) 62–69.
[17] L. Camoglio, A.A. te Velde, A. de Boer, F.J. ten Kate, M. Kopf, S.J. van Deventer, Hapten-induced colitis associated with maintained Th1 and inflammatory responses in IFN-gamma receptor-deficient mice, Eur. J. Immunol. 30 (2000) 1486–1495.
[18] W.I. Khan, P.A. Blennerhasset, A.K. Varghese, S.K. Chowdhury, P. Omsted, Y. Deng, et al., Intestinal nematode infection ameliorates experimental colitis in mice, Infect. Immun. 70 (2002) 5931–5937.
[19] I. Nagano, Z. Wu, Y. Takahashi, Functional genes and proteins of *Trichinella* spp, Parasitol. Res. 104 (2009) 197–207.
[20] P. Jiang, Z.Q. Wang, J. Cui, X. Zhang, Comparison of artificial digestion and Baermann's methods for detection of *Trichinella spiralis* pre-encapsulated larvae in muscles with low-level infections, Foodborne Pathog. Dis. 9 (2012) 27–31.
[21] F. Li, J. Cui, Z.Q. Wang, P. Jiang, Sensitivity and optimization of artificial digestion in the inspection of meat for *Trichinella spiralis*, Foodborne Pathog. Dis. 7 (2010) 879–885.
[22] L. Wang, J. Cui, D.D. Hu, R.D. Liu, Z.Q. Wang, Identification of early diagnostic antigens from major excretory-secretory proteins of *Trichinella spiralis* muscle larvae using immunoproteomics, Parasit. Vectors 7 (2014) 40.
[23] M.B. Lutz, N. Kukutsch, A.L. Ogilvie, S. Rossner, F. Koch, N. Romani, et al., An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow, J. Immunol. Methods 223 (1999) 77–92.
[24] S. Wang, Y. Xie, X. Yang, X. Wang, K. Yan, Z. Zhong, et al., Therapeutic potential of recombinant cystatin from Schistosoma japonicum in TNBS-induced experimental colitis of mice, Parasit. Vectors 9 (2016) 6.
[25] R.D. Liu, Z.Q. Wang, L. Wang, S.R. Long, H.J. Ren, J. Cui, Analysis of differentially expressed genes of *Trichinella spiralis* larvae activated by bile and cultured with intestinal epithelial cells using real-time PCR, Parasitol. Res. 112 (2013) 4113–4120.
[26] J. Cui, H.J. Ren, R.D. Liu, L. Wang, Z.F. Zhang, Z.Q. Wang, Phage-displayed specific polypeptide antigens induce significant protective immunity against *Trichinella spiralis* infection in BALB/c mice, Vaccine 31 (2013) 1171–1177.
[27] J.F. Li, K.X. Guo, X. Qi, J.J. Lei, Y. Han, S.W. Yan, et al., Protective immunity against Trichinella spiralis in mice elicited by oral vaccination with attenuated Salmonella-delivered TsSP1.2 DNA, Vet. Res. 49 (2018) 87.
[28] K.J. Maloy, F. Powrie, Intestinal homeostasis and its breakdown in inflammatory bowel disease, Nature 474 (2011) 298–306.
[29] V. Driss, M. El Nady, M. Delbeke, C. Rousseaux, C. Dubuquoy, A. Sarazin, et al., The schistosome glutathione S-transferase P28GST, a unique helminth protein, prevents intestinal inflammation in experimental colitis through a Th2-type response with mucosal eosinophils, Mucosal Immunol. 9 (2016) 322–335.
[30] R.M. Eichenberger, S. Ryan, L. Jones, G. Buitrago, R. Polster, M. Montes de Oca, et al., Hookworm secreted extracellular vesicles interact with host cells and prevent inducible colitis in mice, Front. Immunol. 9 (2018) 850.
[31] T. Arai, F. Lopes, A. Shute, A. Wang, D.M. McKay, Young mice expel the tapeworm Hymenolepis diminuta and are protected from colitis by triggering a memory response with worm antigen, Am. J. Physiol. Gastrointest. Liver Physiol. 314 (2018) : (G461-G70).
[32] J.W. Williams, M.Y. Tjota, B.S. Clay, B. Vander Lugt, H.S. Bandukwala, C.L. Hrusch, et al., Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation, Nat. Commun. 4 (2013) 2990.
[33] E.J. Villablanca, V. Russo, J.R. Mora, Dendritic cell migration and lymphocyte homing imprinting, Histol. Histopathol. 23 (2008) 897–910.
[34] H. Torres-Aguilar, S.R. Aguilar-Ruiz, G. Gonzalez-Perez, R. Munguia, S. Bajana, M.A. Meraz-Rios, et al., Tolerogenic dendritic cells generated with different immunosuppressive cytokines induce antigen-specific anergy and regulatory properties in memory CD4+ T cells, J. Immunol. 184 (2010) 1765–1775.
[35] S. Rutella, G. Bonanno, A. Procoli, A. Mariotti, D.G. de Ritis, A. Curti, et al., Hepatocyte growth factor favors monocyte differentiation into regulatory interleukin (IL)-10++IL-12low/neg accessory cells with dendritic-cell features, Blood 108 (2006) 218–227.
[36] S. Wirtz, V. Popp, M. Kindermann, K. Gerlach, B. Weigmann, S. Fichtner-Feigl, et al., Chemically induced mouse models of acute and chronic intestinal inflammation, Nat. Protoc. 12 (2017) 1295–1309.
[37] C.M. Lloyd, R.J. Snelgrove, Type 2 immunity: expanding our view, Sci. Immunol. 3 (2018).
[38] K. Guo, X. Sun, Y. Gu, Z. Wang, J. Huang, X. Zhu, Trichinella spiralis paramyosin activates mouse bone marrow-derived dendritic cells and induces regulatory T cells, Parasit. Vectors 9 (2016) 569.
[39] E.M. Shevach, Foxp3(+) T regulatory cells: still many unanswered questions-a perspective after 20 years of study, Front. Immunol. 9 (2018) 1048.
[40] C.J.C. Johnston, D.J. Smyth, R.B. Kodali, M.P.J. White, Y. Harcus, K.J. Filbey, et al., A structurally distinct TGF-beta mimic from an intestinal helminth parasite potently induces regulatory T cells, Nat. Commun. 8 (2017) 1741.